STUDY : Th 472 / 01-2649

BERKEM

ACUTE ORAL TOXICITY TEST IN THE RAT

- Limit test -

CHRYSANTHELLUM

- TEST REPORT -

CONFIDENTIAL

12 pages in this report including 4 in Appendices

Blanquefort, October 17, 2002

PG/MCC

48, rue Jean Duvert - F33290 BLANQUEFORT - Tél. 33 (0)5 56 95 59 95 - Fax 33 (0)5 56 95 05 22 - E-mail : evicfrance-blanquefort@evic.fr
57, rue Ulysse Gayon - F33000 BORDEAUX - Tél. 33 (0)5 57 14 00 80 - Fax 33 (0)5 56 48 72 49 - E-mail : evicfrance-idec@evic.fr
51, avenue de Paris - F94300 VINCENNES - Tél. 33 (0)1 41 74 40 23 - Fax 33 (0)1 41 74 40 24 - E-mail : evicfrance-paris@evic.fr
SA au capital de 305 878 € - RC 70B70 Bordeaux - SIRET 470 200 700 00016 - FR 794 70200700

CONTENTS

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EVALUATION DE LA TOXICITE AIGUE APRES ADMINISTRATION PAR VOIE ORALE CHEZ LE RAT (EPREUVE LIMITE) - LIGNES DIRECTRICES 401 DE L'OCDE (24/2/1987)

ACUTE ORAL TOXICITY TEST IN THE RAT (LIMIT TEST) - OECD GUIDELINE 401 (24/2/1987)

- Substance testée */Test substance* : CHRYSANTHELLUM – lot / *batch* 2890

- **Résumé de l'essai/*Summary of the test :***

L'objectif de l'essai a été d'apprécier qualitativement et quantitativement les phénomènes toxiques et le délai de leur apparition après administration unique de 5000 mg/kg de poids corporel, de la substance à tester diluée dans l'eau PPI, par voie orale, chez 10 rats (5 mâles et 5 femelles).

Les animaux ont été observés quotidiennement pendant 14 jours au moins après l'administration et les signes de toxicité dont la mortalité ont été notés.
La substance testée a été classée selon les critères définis par la Directive de base 67/548/CEE du Journal Officiel des Communautés Européennes du 27 juin 1967 et ses amendements successifs.

The aim of the study was to assess qualitatively and quantitatively the toxic effects and the delay of appearance after single oral administration of 5000 mg/kg of body weight, of test substance diluted with distilled water, in 10 rats (5 males and 5 females).

The animals were daily observed for at least 14 days after administration and the signs of toxicity (mortality...) were noted.
The test substance was classified according to the criteria defined in the basic Directive 67/548/EEC of the Official Journal of the European Communities of June 27, 1967 and its successive amendments.

- **Dates de l'expérimentation/*Experimental dates*** : du/*from* 16/10/01 au/*to* 31/10/01

- **Résultats/*Results* :**

La DL0 et la DL50 sont supérieures à 5000 mg/kg.
The LD0 and the LD50 are higher than 5000 mg/kg.

- **Conclusion/*Conclusion* :**

La substance testée peut être considérée comme une substance qui ne présente pas de risque important de toxicité aiguë si elle est avalée.
The test substance can be considered as a substance which does not present a significant acute toxic risk if swallowed.

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1. AIM AND PRINCIPLE OF THE TEST

The aim of the test is to assess qualitatively and quantitatively the toxic phenomena and the rate of onset after single administration, by oral route in the Rat, of a test substance or preparation.

The test substance or preparation is orally administered at one dose, by gavage, to a group of animals.

The animals are observed each day for 14 days at least, after administration, to detect the signs of toxicity. Animals which die are necropsied ; the animals which are intercurrently sacrificed as well as the ones which survive until the end of the test are necropsied.

Rat is the Rodent species commonly used and recommended by official authorities for the assessment of chemical substances safety by this type of method.

The test allows to classify the test substance according to the criteria defined in the Directive 67/548/EEC and its successive amendments.

2. REFERENCES

- the decree of April 20, 1994 published in the Official Journal of the French Republic of May 08, 1994 taken in enforcement from the basic Directive 67/548/EEC published in the Official Journal of the European Communities of June 27, 1967
- the OECD guideline 401 of February 24, 1987 concerning the tests of chemical products
- the part B1 of the appendix V of the Directive 92/69/EEC of July 31, 1992 published in the Official Journal of the European Communities of December 29, 1992 (L 383A).

3. ETHICS AND APPROVAL OF THE TEST FACILITY

The test was entirely performed in the premises of Evic-Tox Department of EVIC france Company in Blanquefort, according to the animal ethical rules mentioned in the **European Directive 86/609/EEC** of November 24, 1986 and was submitted to the previous agreement of the animal Ethics Committee internal to the Test Facility.

It was conducted according to the internal rules of the Quality System of EVIC france company, which was declared in conformity with the GLP by the **AFSSAPS** (decree of March 14, 2000 published in the Official Journal of the French Republic of March 23, 2000) and the **GIPC** (decree No 98-1312 of December 31, 1998 published in the Official Journal of the French Republic of January 1st, 1999) and with the EN45001 standard by the **COFRAC** (accreditation No 1-0042).

4. QUALITY ASSURANCE

All the data collected during the test were recorded by the technician responsible for the test, on the documents reserved for that effect.

Each page of these documents was initialled and dated by the technician responsible for the test.
Any missing data was justified and the corrections were initialled and dated.

The Quality Assurance Unit ensured by periodic inspections that the study plan and working procedures relevant to this type of test were strictly applied.
The experimental data and the test report were audited in accordance with the procedure implemented in the Test Facility.

At the end of the test, the work documents were filed with the test report for 10 years.
At the end of this period, the Test Facility defines with the Sponsor the carrying out of the filing, the restitution of the data or their destruction.

Drinking : acidified tap water (pH = 2.5) distributed in polypropylene bottles with stainless steel teat. A sample of water was taken after each technical intervention from the pipes and every 6 months at least and sent for chemicophysical and bacteriological analysis to a specialized control organization.

7. TEST PROCEDURE

7.1. Preparation of the test substance

The test substance was administered diluted with distilled water (Cooper – batch 0003) and maintained under magnetic stirring during treatment.

The vehicle was chosen according to the nature of the test substance and producing no painful effect in disagreement with the rules of animal ethic.

The preparation of the substance was performed extemporaneously the day of the test D1/T0 in sufficient quantity for the necessities of the test. The method of preparation was reported in the work document reserved for that effect.

The test substance was brought to the test room according to the modalities defined in the procedure of the Test Facility.

7.2. Administration of the test substance (D1/T0)

The animals, fasted prior to the test substance administration, were weighed again on D1 before administration. The test substance was administered in a single dose, orally, to each animal, by gavage using a syringe with appropriate volume, fitted with a suitable sized canula at the dose of **5000 mg/kg** of body weight.

The volume per kg of body weight, as equal to **10 ml/kg**, the volumes of test solution were calculated for each rat.

After administration, animals were fasted for 3 to 4 hours.

7.3. Body weight

The animals were regularly weighed on D-1 the day before administration then on D1/T0 just before administration of the substance and on D4, D8 and D15 i.e. 3, 7 and 14 days after administration of the test substance.
The results of the weighings were reported in the work document reserved for that effect.

7.4. Clinical observations

Animals were regularly observed the day of administration (immediately, during the 30 minutes following gavage, 1h, 2h, 3h, 4h, 5h, 6h after administration) then at least once a day for 14 days at least.

Just after administration of the test substance, the attention was directed to suffocation signs : in case of mortality, dead animals were necropsied immediately and it was checked that the death was related to the toxicity of the test substance and not to an error of route.

Systematically, since the appearance of the clinical signs and at the latest, 1h after administration, the clinical observations were made individually, each animal being examined outside the home cage.

At the other control times, the examinations were performed cageside, without touching the animals. Any animal which had an abnormal behaviour was taken out from its cage and submitted to an individual examination.

The different parameters observed were the following ones : spontaneous activity, Preyer's reflex, respiratory effect, convulsions, tremors, temperature, muscular tone, grip strength, palpebral ptosis, mydriasis, salivation, lacrimation, turnaround reflex, piloerection, diarrhoea, lethargy, coma, changes in skin, fur, eyes, mucous membranes and death.

The results of the clinical observations were registered in the work document reserved for that effect.

7.5. Necropsy

All the animals surviving at the end of the 14 days of observation were sacrificed on D15 by intraperitoneal injection of a 6 % sodium Pentobarbital® solution at the rate of 1.16 ml/kg and bled at the femoral artery. They were necropsied and the main organs were examined macroscopically.

All the pathological changes were recorded in the document reserved for that effect.

7.6. Interpretation of the results

The assessment criteria of the toxicity of the test substance taken into account were :
- body weight change
- clinical and behavioural signs
- necropsy findings
- the mortality expressed in percentage of compound-related deaths.

If no death was noted and if the clinical examinations, the necropsy findings and the body weight change were normal, we were able to consider the test substance as harmless if swallowed.

8. RESULTS

The individual weights of the animals assessed during the test are supplied in Appendix I.

The results of the clinical observations are summarized in the table enclosed in Appendices II.

The results of the necropsy findings are summarized in the table enclosed in Appendix III.

9. CONCLUSION

Taking into account the criteria defined by the Directive 67/548/EEC and its successive amendments, the substance **CHRYSANTHELLUM** can be considered as a substance which **does not present a significant acute toxic risk if swallowed.**

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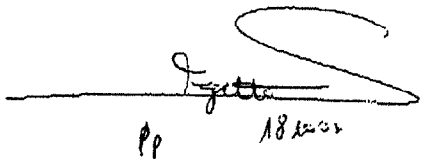
10. STUDY RESPONSIBLE PERSONNEL'S STATEMENT

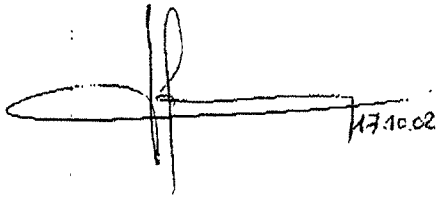
Test Facility Management

I the undersigned, **Philippe MASSON**, declare to have designated **Patrick GOMOND** as the Study Director and ensured that he approved the study plan with full knowledge of the facts and made it available to the Quality Assurance Unit.



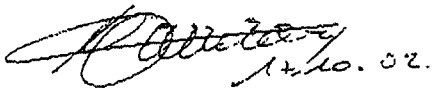
Study Director

I the undersigned, **Patrick GOMOND**, declare that the overall conduct of the study was carried out under my responsibility and in accordance with the principles of Good Laboratory Practices according to the rules appropriate to this study



Quality Assurance

I the undersigned, **Michèle DARRICAU**, declare that :
- this kind of study was inspected according to the procedure of the Test Facility on October 26, 2001,
- the report of the inspection was transmitted to the Management and to the Study Director on October 29, 2001,
- the final report was audited on November 15, 2001 and this report was examined on October 17, 2002,
- the results reported accurately and completely reflect the raw data of the study.



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Appendix I

Body Weight

Male rats

Animals No	Weight (in g)				
	D1	D4	D8	D15	D15-D1
7166	209.5	229.7	259.6	300.6	91.1
7167	196.4	243.8	283.2	331.5	135.1
7168	185.6	245.9	280.7	330.0	144.4
7169	186.3	225.6	254.8	298.8	112.5
7170	199.5	229.6	260	330.7	131.2
Mean	195.5	234.9	267.7	318.3	122.9
standard deviation	9.9	9.2	13.2	17.0	21.2

Female rats

Animals No	Weight (in g)				
	D1	D4	D8	D15	D15-D1
7171	183.6	208.0	218.4	227.9	44.3
7172	172.1	205.9	211.5	222.4	50.3
7173	176.9	215.9	222.6	223.3	46.4
7174	169.4	207.8	215.9	227.1	57.7
7175	192.4	202.3	207.3	213.4	21.0
Mean	178.9	208.0	215.1	222.8	43.9
standard deviation	9.3	5.0	5.9	5.8	13.8

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Appendix II/1

Clinical observations

Male rats

Observation time	Comments	Observation time	Comments
D1 (after treatment)	NTR	D5	NTR
D1 T30'	NTR	D6	NTR
D1 T1h	NTR	D7	NTR
D1 T2h	NTR	D8	NTR
D1 T3h	NTR	D9	NTR
D1 T4h	NTR	D10	NTR
D1 T5h	NTR	D11	NTR
D1 T6h	NTR	D12	NTR
D2	NTR	D13	NTR
D3	NTR	D14	NTR
D4	NTR	D15	NTR

Legend : NTR : nothing to report

Annexe II/2

Observations cliniques
Rats Femelles

Temps d'observation	Commentaires	Temps d'observation	Commentaires
J1 (après traitement)	RAS	J5	RAS
J1 T30'	RAS	J6	RAS
J1 T1h	RAS	J7	RAS
J1 T2h	RAS	J8	RAS
J1 T3h	RAS	J9	RAS
J1 T4h	RAS	J10	RAS
J1 T5h	RAS	J11	RAS
J1 T6h	RAS	J12	RAS
J2	RAS	J13	RAS
J3	RAS	J14	RAS
J4	RAS	J15	RAS

Appendix III

Necropsy

Male rats

Animals No	Death		Comments
	Day	Reason	
7166	D15	S	NTR
7167	D15	S	NTR
7168	D15	S	NTR
7169	D15	S	NTR
7170	D15	S	NTR

Female rats

Animals No	Death		Comments
	Day	Reason	
7171	D15	S	NTR
7172	D15	S	NTR
7173	D15	S	NTR
7174	D15	S	NTR
7175	D15	S	NTR

Legend : *NTR = nothing to report*
ND = natural death
S = sacrifice
AD = accidental death

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OECD GUIDELINE FOR TESTING OF CHEMICALS

Adopted by the Council on 17th July 1992

Acute Oral Toxicity - Fixed Dose Method

INTRODUCTION

1. Traditional methods for assessing acute oral toxicity, like Guideline 401, use death of animals as an endpoint. In 1984, a new approach to acute toxicity testing was suggested by the British Toxicology Society (BTS) based on a fixed-dose procedure (1). This avoided using death of animals as an endpoint, and relied instead on the observation of clear signs of toxicity developed at one of a series of fixed dose levels. This theoretical approach by the BTS was subjected to a validation study in the UK (2). Subsequently an international validation study, sponsored by the Commission of European Communities and UK Government Departments, was carried out under the patronage of the OECD (3). Both studies showed that the procedure was scientifically valid.

2. The fixed dose method set out in this guideline provides information both for hazard assessment purposes and for ranking substances. A preliminary sighting study, using a small number of animals, is included in this guideline in order to estimate the dose-effect for toxicity and mortality and to provide information on dose selection for the main study. Results from the sighting and main studies enable compounds to be ranked in different classification systems, currently in use.

3. Definitions used are set out in Annex 1.

INITIAL CONSIDERATIONS

4. It is a principle of the fixed dose method that in the main study only moderately toxic doses are used, and that administration of doses that are expected to be lethal should be avoided. Also doses that are known to cause marked pain and distress, due to corrosive or severely irritant actions, need not be administered. During the test, animals obviously in pain or showing signs of severe distress should be humanely killed.

PRINCIPLE OF THE TEST

5. In a preliminary sighting study, the effects of various doses administered to single animals of one sex are investigated in a sequential manner. The sighting study yields information on the dose-toxicity relationship, including an estimate of the minimum lethal dose. In the main study, the substance is administered to groups of 5 male and 5 female animals at one of the fixed doses (5, 50, 500, and 2000 mg/kg). The dose is derived from the sighting study. It is immediately below that which is expected to result in mortality. If evident toxicity is not seen at the chosen dose level, the substance should be retested at the next higher dose level. If, at the initial dose level, animals die, or a severe toxic reaction requires the removal of animals from the study for animal welfare reasons, the substance is retested at the next lower dose level. Consideration of data from both the sighting study

and the main study will enable a judgement to be made as to whether the interpretation set out in Annex 2 (and the note thereto) is acceptable, or whether some adjustment is needed.

DESCRIPTION OF THE METHOD

Selection of animal species

6. The rat is the preferred species. Commonly used laboratory strains should be employed. The weight variation in animals used in a test should not exceed ± 20 per cent of the mean weight. The females should be nulliparous and non-pregnant.

Housing and feeding conditions

7. The temperature of the experimental animal room should be 22°C (±3°C) and the relative humidity 30-70 per cent. Animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g. morbidity, excitability) may indicate a need for individual caging. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

Preparation of the animals

8. Healthy young adult animals are randomly selected and acclimatised to the laboratory conditions for at least 5 days prior to the test.

Preparation of doses

9. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that wherever possible the use of an aqueous solution be considered first, followed by consideration of a solution in oil (e.g. corn oil) and then by possible solution in other vehicles. For non-aqueous vehicles the toxic characteristics of the vehicle should be known, and if not known should be determined before the test.

Administration of doses

10. The test substance is administered in a single dose to the animals by gavage using a stomach tube or a suitable intubation cannula. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not exceed 1 ml/100g body weight, except in the case of aqueous solutions where 2 ml/100g body weight may be used. Variability in test volume should be minimised by adjusting the concentration to ensure a constant volume at all dose levels. If a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

11. Animals should be fasted prior to substance administration by withholding food over-night. Following the period of fasting, the animals should be weighed and then the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hours. Where a dose is administered in fractions over a period it may be necessary to provide the animals with food and water depending on the length of the period.

PROCEDURE

Sighting Study

12. The effects of various doses are investigated in single animals. Normally female animals will be used in the absence of information derived from structure-activity relationships or other information indicating that males will be the more sensitive sex. Dosing is sequential, allowing at least 24 hours before dosing the next animal. All animals are carefully observed for signs of toxicity for at least 7 days; if signs of moderate toxicity persist at 7 days, the animal should be observed for up to an additional 7 days. The following initial dose levels are considered: 5, 50, 500 and 2000 mg/kg. If the initial dose chosen does not produce severe toxicity, and the next higher level produces mortality, then it will be necessary to investigate one or more intermediate dose levels as appropriate. In this way it should be possible to build up information on the dose level(s) that produce(s) some signs of toxicity and the minimum dose level that produces mortality.

13. An effort should be made to select the initial dose using evidence from related chemicals. In the absence of such information, it is suggested that the 500 mg/kg dose is used in the first instance. If no signs of toxicity are seen at the initial dose, then the next higher dose level is investigated. If no mortality occurs at 2000 mg/kg, the sighting test is complete and the main study should be conducted at this dose level. If severe effects, necessitating humane killing are seen at the initial dose (e.g. 500 mg/kg), the next lower dose (e.g. 50 mg/kg) is given to another animal. If this animal survives, further animals may then be dosed with the appropriate intermediate dose levels beween the fixed doses. Normally, one would not expect to use more than 5 animals in this procedure.

Main Study

Number of animals and dose levels

14. At least 10 animals (5 male and 5 female) should be used for each dose level investigated. The dose level to be used in the main study is selected from one of four levels, 5, 50, 500 and 2000 mg/kg body weight and should be that which is judged on the basis of the results from the sighting study likely to produce evident toxicity but no mortality. If the data from the sighting study suggest that mortality will occur at 5 mg/kg, the substance can be investigated at a lower dose level.

15. In most cases it is likely that the data obtained from the sighting study will be adequate to allow the appropriate dose level to be selected in the main study (i.e. a level that produces evident toxicity but no mortality). However if evident toxicity is not seen at the initial dose level, the substance should be retested at the next higher level. If animals die at the initial dose chosen, or a severe reaction requires removal of animals from the study for animal welfare reasons, the substance should be retested at the next lower dose level.

Observations

16. Except where animals need to be removed from the study and humanely killed for animal welfare reasons, animals should be observed for at least 14 days after dosing. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate to onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Clinical examinations

17. A careful clinical examination should be made at least twice on the day of dosing and once

each day thereafter. Animals obviously in pain or showing severe signs of distress should be humanely killed. Additional observations may be necessary during the first few days after dosing so that the test may be terminated if it becomes apparent that the initial dose level chosen was too high. Cageside observations should include changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. When animals are killed for humane reasons or are found dead, the time of death should be recorded as precisely as possible. Individual weights of animals should be determined shortly before the test substance is administered, weekly thereafter and at death. Weight changes should be calculated and recorded. At the end of the test surviving animals are weighed and then sacrificed.

Pathology

18. All test animals (including those which die during the test or are removed from the study for animal welfare reasons) should be subjected to gross necropsy. All gross pathological changes should be recorded. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours should also be considered because it may yield useful information.

DATA AND REPORTING

Data

19. All data from both the sighting and main studies should be summarised in tabular form showing for each dose level tested the number of animals used; the number displaying signs of toxicity; the number of animals found dead during the test or killed for humane reasons; a description of the toxic effects, and in the case of the main study whether evident toxicity was observed; the time course of any toxic effects; and the necropsy findings. General guidance on the interpretation of the results is given in Annex 2 and the note thereto.

Test report

20. The test report must include the following information for both the sighting and main studies, as appropriate:

Test substance:

- physical nature and, where relevant, physicochemical properties;
- identification data.

Vehicle:

- justification for choice of vehicle.

Test animals:

- species/strain used;
- number, age and sex of animals;
- source, housing conditions, diet, etc.;
- individual weights of animals at the start of the test.

Test conditions

Results:

- tabulation of response data by sex and dose level (i.e., number of animals used, number showing signs of toxicity including mortality, nature, severity and duration of effects);
- time course of onset of signs of toxicity and whether these were reversible;
- necropsy findings and any histopathological findings;
- the rationale for identification of the dose level that produced evident toxicity.

Discussion and interpretation of results, including the estimated minimum lethal dose, and the highest dose level that did not produce mortality.

LITERATURE

(1) British Toxicology Society Working Party on Toxicity (1984). A new approach to the classification of substances and preparations on the basis of their acute toxicity. Human Toxicol, 3, 85-92.

(2) Van den Heuvel M.J., Dayan A.D and Shillaker R.D. (1987). Evaluation of the BTS approach to the testing of substances and preparations for their acute toxicity. Human Toxicol, 6, 279-291.

(3) Van den Heuvel M.J., Clark D.G., Fielder R.J., Koundakjian P.P., Oliver G.J.A., Pelling D., Tomlinson N.J. and Walker A.P. (1990). The international validation of a fixed-dose procedure as an alternative to the classical LD50 test. Fd Chem. Toxic, 28, 469-482.

ANNEX 1

DEFINITIONS

Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours.

Dose is the amount of test substance administered. Dose is expressed as weight (g, mg) or as weight of test substance per unit weight of test animal (e.g. mg/kg).

Dosage is a general term comprising the dose, its frequency and the duration of dosing.

Evident toxicity is a general term describing clear signs of toxicity following administration of test substance. These should be sufficient for hazard assessment and, in relation to the fixed dose procedure, should be such that an increase in the dose administered can be expected to result in the development of severe toxic signs and probable mortality.

ANNEX 2

INTERPRETATION OF RESULTS

DOSE	RESULTS	INTERPRETATION
5 mg/kg*	less than 100% survival**	Compounds which may be very toxic (i.e. with LD50 values of ca 25 mg/kg or less) if swallowed.
	100% survival, but evident toxicity	Compounds which may be toxic (i.e. with LD50 values between ca 25 mg/kg and ca 200 mg/kg) if swallowed.
	100% survival, no evident toxicity	Test at 50 mg/kg if not already tested at that dose.
50 mg/kg	less than 100% survival**	Compounds which may be toxic or very toxic if swallowed. Test at 5 mg/kg if not already tested at that dose level.
	100% survival, but evident toxicity	Compounds which may be harmful (i.e. with LD50 values between ca 200 mg/kg and 2000 mg/kg) if swallowed.
	100% survival, no evident toxicity	Test at 500 mg/kg if not already tested at that dose level.
500 mg/kg	less than 100% survival**	Compounds which may be toxic or harmful if swallowed. Test at 50 mg/kg if not already tested at that dose level.
	100% survival, but evident toxicity	Compounds with LD50 values above ca 2000 mg/kg but which may be of some concern due to the nature of the toxic effects.
	100% survival, no evident toxicity	Test at 2000 mg/kg if not already tested at that dose level.
2000 mg/kg	less than 100% survival**	Compounds which may be of some concern if swallowed. Test at 500 mg/kg if not already tested at that dose level.
	100% survival, with or without evident toxicity	Compounds which do not present a significant acute toxic risk if swallowed.

* Where a dose of 5 mg/kg produces significant mortality, or where a sighting study suggests that mortality will result at that dose level, the substance should be investigated at a lower dose level. The level chosen should be that which is likely to produce evident toxicity but no mortality.

** Includes compound related mortality and humane kills but not accidental deaths.

Note

Interpretation has been given with respect to the data obtained in the extensive validation studies of the method and relates to approximate LD50 values in the range below 25 mg/kg, 25-200 mg/kg, 200-2000 mg/kg and above 2000 mg/kg. The results can however be used for other ranges by consideration of both the data from the sighting study and the main study, with judgement as to whether the interpretation given here is adequate (bearing in mind the natural variability in the LD50 value and of the slope of the dose-response curve) or whether any adjustment is necessary.